

Rec'd PCT/PTO 27 JAN 2005

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
29 April 2004 (29.04.2004)

PCT

(10) International Publication Number
WO 2004/034966 A2

(51) International Patent Classification⁷:

A61K

(21) International Application Number:

PCT/US2003/024625

(22) International Filing Date: 6 August 2003 (06.08.2003)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/401,652 6 August 2002 (06.08.2002) US

(71) Applicant (for all designated States except US): LOMA
LINDA UNIVERSITY [US/US]; 2488 Prospect Street,
Loma Linda, CA 92354 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): ESCHER, Alan, P.
[US/US]; 463 Jefferson Street, Redlands, CA 92374 (US).
LI, Fengchun [CN/US]; 24530 University Avenue, Apt.
10, Loma Linda, CA 92354-2732 (US).

(74) Agents: FARAH, David, A. et al.; Sheldon & Mak, PC,
225 South Lake Avenue, 9th Floor, Pasadena, CA 91101
(US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC,
SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA,
UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

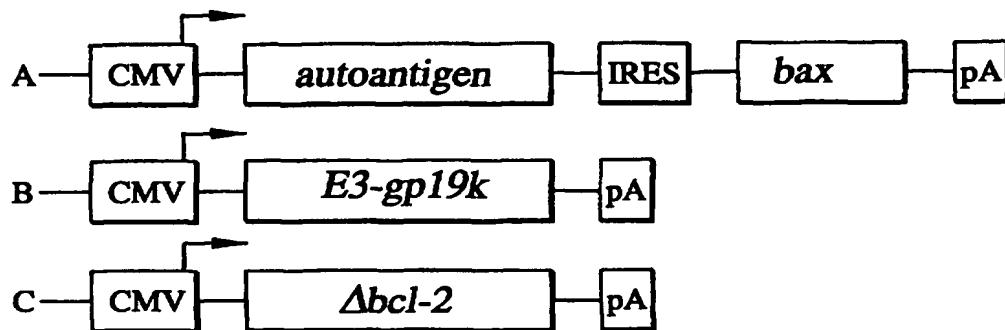
(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished
upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: SUBSTANCES FOR PREVENTING AND TREATING AUTOIMMUNE DISEASES



WO 2004/034966 A2

(57) Abstract: A substance for preventing, delaying the onset of, or treating one or more than one autoimmune disease, the substance comprising a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for the autoimmune disease. A method for preventing, delaying the onset of or treating an autoimmune disease in a patient comprising selecting a patient who is susceptible to developing the autoimmune disease, who is developing the autoimmune disease or who has the autoimmune disease and administering to the patient one or more than one dose of a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for an autoimmune disease, or comprising a polynucleotide sequence encoding the adenoviral protein E3-GP19k, or comprising a polynucleotide sequence encoding ΔBCL-2.

SUBSTANCES FOR PREVENTING AND TREATING AUTOIMMUNE DISEASES**STATEMENT REGARDING FEDERALLY SPONSORED
RESEARCH OR DEVELOPMENT**

This invention was made with United States Government support under Cooperative Agreement Number DAMD-17-97-2-7016 with the National Medical Technology Testbed, Inc., United States Department of the Army. The United States Government has certain rights in this invention.

CROSS-REFERENCE TO RELATED APPLICATION

The present Application claims the benefit of United States provisional patent application 60/401,652, titled "Method and Substances for the Suppression of Diabetes," filed August 6, 2002, the contents of which are incorporated in this disclosure by reference in their entirety.

BACKGROUND

Autoimmune diseases cause significant human morbidity and mortality. These diseases include approximately 80 diseases, such as rheumatoid arthritis, systemic lupus and multiple sclerosis, and affect approximately 5% of the population of the United States. One autoimmune disease, type 1 diabetes, is the most frequent chronic disease in children, and has a steadily increasing worldwide incidence.

Generally, the onset of type 1 diabetes begins with the display by antigen presenting cells (APCs) of autoantigens synthesized by pancreatic beta cells. This display results in the immune system destruction of pancreatic beta cells mediated mostly by T helper 1 (Th1) and cytotoxic T lymphocytes and, thereby, to the loss of insulin production.

Many prophylactic and therapeutic approaches for type 1 diabetes attempt to prevent the destruction of beta cells by inducing the immune system to delete, inactivate or suppress pathogenic self-reactive lymphocytes, such as by administering vaccines that solely deliver autoantigen, or by administering substances are direct effectors of the immune system, such as cytokines. However, currently available DNA-based vaccines are not completely efficient in preventing the disease, and the use of some of these vaccines are associated with inducing or enhancing autoimmunity rather than preventing the disease. Additionally, the use of cytokines is associated with significant morbidity.

Therefore, there is a need for a new method for preventing, delaying the onset of, or

treating autoimmune diseases using vaccines that are not associated with these disadvantages. Further, there is a need for a new method for preventing, delaying the onset of, or treating type 1 diabetes using vaccines that are not associated with these disadvantages.

SUMMARY

5 According to one embodiment of the present invention, there is provided a substance for preventing, delaying the onset of or treating one or more than one autoimmune disease. The substance comprises a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for the autoimmune disease.

10 According to another embodiment of the present invention, there is provided a use of a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for an autoimmune disease for the manufacture of a medicament for preventing, delaying the onset of or treating the one or more than one autoimmune disease.

15 According to another embodiment of the present invention, there is provided a use of a polynucleotide construct comprising a polynucleotide sequence encoding the adenoviral protein E3-GP19k for the manufacture of a medicament for preventing, delaying the onset of or treating one or more than one autoimmune disease.

20 According to another embodiment of the present invention, there is provided a use of a polynucleotide construct comprising a polynucleotide sequence encoding ΔBCL-2 for the manufacture of a medicament for preventing, delaying the onset of or treating one or more than one autoimmune disease.

25 In one embodiment, the medicament is manufactured in dosage units of between about 0.5 mg to about 5 mg. In another embodiment, the medicament is manufactured in dosage units of between about 1 mg to about 4 mg. In another embodiment, the medicament is manufactured in dosage units of between about 2.5 mg to about 3 mg. In another embodiment, the medicament is manufactured in a form suitable for intramuscular administration. In another embodiment, the medicament is manufactured in a form suitable for intravenous administration.

30 According to another embodiment of the present invention, there is provided a method for preventing, delaying the onset of or treating an autoimmune disease in a patient. The method comprises selecting a patient who is susceptible to developing the autoimmune

disease, who is developing the autoimmune disease or who has the autoimmune disease; and administering to the patient one or more than one dose of a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for the autoimmune disease, or a polynucleotide construct comprising a polynucleotide sequence encoding the adenoviral protein E3-GP19k, or a polynucleotide construct comprising a polynucleotide sequence encoding ΔBCL-2, or a combination of the preceding polynucleotide constructs.

In one embodiment, the autoimmune disease is type I diabetes. In another embodiment, selecting the patient comprises identifying in the patient the presence of anti-insulin or anti-GAD autoantibodies or both anti-insulin and anti-GAD autoantibodies. In another embodiment, selecting the patient comprises identifying in the patient the presence of increasing hyperglycemia. In another embodiment, selecting the patient comprises identifying in the patient the presence of glycosuria. In another embodiment, selecting the patient comprises identifying in the patient the presence of a genetic predisposition to the autoimmune disease.

In another embodiment, the one or more than one dose is a plurality of doses. In another embodiment, administering to the patient one or more than one dose comprises injecting the patient intramuscularly with the one or more than one dose. In another embodiment, the method further comprises, after administering, monitoring the patient for the development the autoimmune disease.

FIGURES

These and other features, aspects and advantages of the present invention will become better understood with regard to the following description, appended claims, and accompanying figures where:

Figure 1 are schematic depictions of three substances according to the present invention; and

Figure 2 are schematic depictions of the fifteen plasmids that were tested for their efficiency in preventing, delaying the onset of or treating an autoimmune disease in accordance with a method of the present invention.

DESCRIPTION

According to one embodiment of the present invention, there are provided substances for preventing, delaying the onset of or treating one or more than one autoimmune disease.

According to another embodiment of the present invention, there is provided a method of preventing, delaying the onset of or treating one or more than one autoimmune disease. In one embodiment, the autoimmune disease is type 1 diabetes. In a preferred embodiment, the method comprising using a substance according to the present invention is a vaccine. The 5 substances and method of the present invention do not use solely the delivery of autoantigen, and do not use molecules that are direct effectors of the immune system as in prior methods. Instead, the present invention uses a vaccine to prevent apoptosis of one or more than one type of cell capable of suppressing the autoimmune disease. Because these one or more than one type of cell capable of suppressing the autoimmune disease are still be subject to 10 physiological and immune regulation, the risk of inducing or enhancing autoimmunity is greatly reduced by the present method as compared to some prior art methods. Further, because the present invention does not involve administering substances that are direct effectors of the immune system, such as cytokines, the present invention does not pose the risk side effects associated with such direct effectors of the immune system. Further 15 advantageously, a genetic vaccine comprising primarily plasmid DNA can be produced in large quantities at relatively low cost and does not require a "cold chain" for storage. Therefore, the substances and methods according to the present invention are both economical and practical for use to prevent, delay the onset of or treat an autoimmune disease. Further, a genetic vaccine according to the present invention modifies the genetic 20 material of an organism directly which means that native epitopes will be processed by the organism's immune system unlike protein-based vaccines. The substances and method of the present invention will now be disclosed in detail.

As used in this disclosure, the term "autoimmune disease" comprises both diseases due in part or in total to destruction of normal cells or tissues by the organism's own immune 25 system, and also comprises destruction of cells or tissues that were transplanted into the organism to take the place of defective or absent cells or tissues, such as islet cell transplants, or partial or whole organ transplants, by the organism's own immune system.

As used in this disclosure, the term "comprise" and variations of the term, such as "comprising" and "comprises," are not intended to exclude other additives, components, 30 integers or steps.

In one embodiment, the present invention includes three substances that can be used either individually, sequentially or simultaneously to prevent, delay the onset of or treat one

or more than one autoimmune disease. One of the three substances is a DNA construct comprising a polynucleotide sequence, SEQ ID NO:1, encoding the pro-apoptotic protein BAX, and encoding one or more than one autoantigen for the autoimmune disease. Another of the three substances is a DNA construct comprising a polynucleotide sequence, SEQ ID NO:2, encoding the adenoviral protein E3-GP19k, which prevents presentation of an antigen on MHC-I molecules in the endoplasmic reticulum. Another of the three substances is a DNA construct comprising a polynucleotide sequence, SEQ ID NO:3, encoding a truncated form of BCL-2 designated ΔBCL-2 in this disclosure.

As will be understood by those with skill in the art with reference to this disclosure, though specific sequences are given for the polynucleotide sequences as disclosed in this disclosure, such as the polynucleotide sequences encoding the pro-apoptotic protein BAX, the adenoviral protein E3-GP19k and ΔBCL-2, the present invention includes any other sequence that does not cause a change in the translated amino acid sequence, as well as any sequence that does cause a change in the translated amino acid sequence but where the change does not substantially affect the function of the translated amino acid sequence so as to make it unsuitable for the uses contemplated in this disclosure.

Referring now to Figure 1, there are shown schematic depictions of three substances according to the present invention. As can be seen, each substance comprises a plasmid DNA construct. Substance A comprises a plasmid construct comprising a polynucleotide encoding an autoantigen for the autoimmune disease, such as secreted glutamic acid decarboxylase that is an autoantigen for type 1 diabetes, followed by a polynucleotide, SEQ ID NO:1, encoding BAX. Substance B comprises a plasmid construct comprising a polynucleotide, SEQ ID NO:2, encoding E3-GP19k without a polynucleotide encoding an autoantigen for the autoimmune disease. Substance C comprises a plasmid construct comprising a polynucleotide, SEQ ID NO:3, encoding a truncated form of the anti-apoptotic protein BCL-2 without a polynucleotide encoding an autoantigen for the autoimmune disease. As used in the Figures, "CMV" represents the cytomegalovirus promoter element, "pA" represents a polyadenylation site, and "IRES" represents an internal ribosome binding site from the EMCV virus, SEQ ID NO:4.

In order to demonstrate the advantages of the present invention, fifteen plasmids were constructed and used as vaccines. Each construct was cloned into the vector pND2. Referring now to Figure 2, there are shown schematic depictions of the fifteen plasmids that

were tested for their efficiency in preventing, delaying the onset of or treating an autoimmune disease. As can be seen, each plasmid was under the plasmid transcriptional control of the same promoter (CMVp) to ensure expression of both open reading frames in each transfected cells. During construction of these plasmids containing the cDNA encoding BCL-2, it was
5 found that plasmid deletions occurred due to the large size of the cDNA. Therefore, a truncated version of *bcl-2* designated $\Delta bcl-2$ was used to construct the plasmids. As shown in Figure 2, the plasmids comprised cDNA encoding cytoplasmic GAD, SEQ ID NO:5, (plasmid 1); secreted GAD (SGAD), SEQ ID NO:6, (plasmid 2); a control secreted luciferase, SEQ ID NO:7, (plasmid 3); truncated human anti-apoptotic protein BCL-2
10 (Δ BCL-2), SEQ ID NO:3, (plasmid 4); anti-apoptotic protein BAX, SEQ ID NO:1, (plasmid 5); E3-GP19k, SEQ ID NO:2, (plasmid 6); Δ BCL-2, SEQ ID NO:3, in combination with cytoplasmic GAD, SEQ ID NO:5, secreted GAD, SEQ ID NO:62, and secreted luciferase, SEQ ID NO:7, (plasmids 7-9, respectively), BAX, SEQ ID NO:1, in combination with cytoplasmic GAD, SEQ ID NO:5, secreted GAD, SEQ ID NO:6, and secreted luciferase,
15 SEQ ID NO:7, (plasmids 10-12, respectively); and E3-GP19k, SEQ ID NO:2, in combination with cytoplasmic GAD, SEQ ID NO:5, secreted GAD, SEQ ID NO:6, and secreted luciferase, SEQ ID NO:7, (plasmids 13-15, respectively).

All plasmids were generated, the open reading frame amplified using PCR, and the amplification products were inspected after DNA sequencing and found to be without
20 mutations. Each construct was then used to transfect simian COS-7 cells transiently for immunoblot analysis of cell lysates, which confirmed that a gene product of the correct size was encoded (data not shown).

Next, the effects of the 15 plasmids on non-obese diabetic (NOD) mice were determined as follows. First, plasmid DNA was isolated using Qiagen Endofree kits (Qiagen
25 Inc., Chatsworth, CA, US), and 300 ug of each of the 15 plasmid DNAs was injected intramuscularly into groups of fifteen 4-5-week-old female NOD mice. The 300 ug dose was selected as a dose relevant to the human clinical setting based on organism weight. The onset of diabetes was monitored until the age of 35 weeks, using urine and blood glucose analysis. The mice were considered diabetic after testing positive for high levels of glycosuria, with
30 blood glucose levels greater than 300 mg/dl on two consecutive days.

The results of these experiments demonstrated the following. The percentage of diabetic animals at 35 weeks of age ranged from 73-93 % for mice vaccinated with plasmids

1-3; 60-67% for mice vaccinated with plasmids 4 or 7-9; 47-85% for mice vaccinated with plasmids 5 and 10-12; and 53-73% for mice vaccinated with plasmids 6 and 13-15. Control animals (those not vaccinated) had an incidence of diabetes of about 93%. Therefore, administration of 300 ug of plasmid vector alone or of 300 ug of plasmid vector encoding 5 antigens alone, plasmids 1-3, did not result in significant diabetes suppression. Mice vaccinated with plasmids 6-9 and 11 showed statistically significant suppression of diabetes when compared to untreated mice ($P < 0.05$ for plasmid 7, and $P < 0.02$ for plasmid 9). In addition, mice receiving pND2-E3-GP19k, plasmid 6 or pND2-SGAD55-BAX, plasmid 11 showed a significantly decreased incidence of diabetes at 35 weeks when compared to mice 10 receiving plasmid pND2-GAD65, plasmid 1 or pND2-GAD65-BAX, plasmid 10 ($P < 0.04$), and mice receiving pND2-GAD65- Δ BCL2, plasmid 7 or pND2-SGAD55- Δ BCL2, plasmid 8 showed significantly decreased diabetes when compared to mice receiving pND2-GAD65, plasmid 1 ($P < 0.05$). Suppression of diabetes was associated with decreased islet inflammation (data not shown). These results will be disclosed now in greater detail.

15 Mice that were vaccinated with plasmids comprising *Abcl-2*, plasmids 4 and 7-9, showed a 4-5 weeks delay in diabetes onset regardless of the co-expressed antigen, and a decrease in the incidence of diabetes at 35 weeks of age (60-67% compared to about 93% for the unvaccinated control mice) regardless of the co-expressed antigen. Therefore, co-expression of GAD autoantigen did not suppress the effect.

20 Mice that were vaccinated with plasmids comprising *bax*, plasmids 5 and 10-12, did not show diabetes suppression, with the exception of *sgad55-bax*, plasmid 11. While mice vaccinated with plasmid 11 started to develop diabetes at a time similar to other mice vaccinated with a plasmid comprising only *bax*, plasmid 5, the incidence of diabetes in mice vaccinated with plasmid 11 at 35 weeks of age was only 47% compared with a 93% 25 incidence for the unvaccinated control mice ($p < 0.05$).

Mice that were vaccinated with plasmids comprising *E3-gp19k*, plasmids 6 and 13-15 showed wide differences in diabetes onset, depending on the antigen that was co-expressed. Mice that were vaccinated with the plasmid comprising *E3-gp19k* without autoantigen, plasmid 6 started to develop diabetes with a 4-5 week delay, and showed decreased diabetes 30 at 35 weeks of age (53% vs 93% for the unvaccinated control mice for control) ($p < 0.05$). Mice that were vaccinated with the plasmids comprising *E3-gp19k* with autoantigen, plasmids 13-15, suppressed the effect, both with respect to the delay in the onset of diabetes and with

respect to the incidence of diabetic animals at 35 weeks.

Next, immune responses were characterized using a GAD-specific ELISpot assay and ELISA of serum anti-GAD IgG isotypes to determine whether diabetes suppression by the administration of the substances of the present invention was associated with suppression of inflammatory Th1-like activity, and up-regulation of anti-inflammatory Th2 like response.

The ELISpot assay was conducted as follows. Splenocytes were isolated from the mice at time of diabetes onset, or at the end of the observation period for non-diabetic animals. The cells were then stimulated with recombinant GAD protein, and the number of cells secreting IFN-gamma (for Th1-like activity), and IL-4 (for Th2-like activity) were counted, following a standard manufacturer's protocol. The number of cells secreting the cytokines in the absence of GAD stimulation was then subtracted, and results analyzed. For IFN-gamma the data clearly indicated that suppression of diabetes by plasmid 6, encoding E3-GP19k alone, or by plasmids 4 and 7-9, encoding Δ BCL-2 alone or together with an antigen, were associated with a suppression of GAD-specific activity. Therefore, E3-19k and Δ BCL-2 could induce an immune response that was able to suppress autoreactivity against beta cells. Surprisingly, the SGAD55-BAX combination did not appear to significantly suppress Th1-like activity. Further, SGAD55 alone, which did not suppress diabetes, did suppress GAD-specific Th1-like response.

With respect to IL-4, the data indicated an increase in GAD-specific activity for mice that received plasmid 6 encoding E3-GP19k alone (diabetes suppression), plasmid 13 encoding SGAD55 and E3-19k (no diabetes suppression), and plasmid 8 SGAD55 and Δ BCL-2(diabetes suppression). Thus, increased Th2-like activity was not always associated with decreased Th1-like activity or disease suppression.

The ELISA was conducted as follows. Animal sera were used for ELISA of anti-GAD IgG2a,b and IgG1 isotypes, which indicate a Th1-like and Th2-like activity, respectively. ELISA of anti-GAD IgG2a,b indicated that three of the plasmid DNAs coding for Δ BCL-2, plasmids 4, 8 and 9, showed a significant reduction in Th1-like activity, when compared to plasmid 5 coding for BAX, but not with the unvaccinated control mice. ELISA of anti-GAD IgG1 indicated that all plasmid DNAs encoding BAX, plasmids 5 and 10-12, resulted in decreased Th2-like activity.

These data taken together indicate that, first, *bax*, a plasmid cDNA coding for a pro-apoptotic protein, can be used as a molecular adjuvant for genetic vaccines to prevent

autoimmune disease, such as a vaccine comprising a polynucleotide encoding a secreted form of an autoantigen. Second, a plasmid cDNA encoding E3-GP19k or encoding a truncated BCL-2 alone could suppress autoimmune disease, though a plasmid cDNA encoding E3-GP19k or encoding a truncated BCL-2 combined with an autoantigen was less effective.

5 In one embodiment of the present invention, there is provided a method of preventing, delaying the onset of or treating an autoimmune disease. The method comprises, first, selecting a patient who is susceptible to developing the autoimmune disease, who is developing the autoimmune disease or who has the autoimmune disease. The selection can be made using standard methods as will be understood by those with skill in the art with
10 reference to this disclosure. For example, if the autoimmune disease is diabetes, the selection can be made by identifying in the patient the presence of anti-insulin or anti-GAD autoantibodies or both anti-insulin and/or anti-GAD autoantibodies, the presence of increasing hyperglycemia, the presence of glycosuria, the presence of a genetic predisposition to diabetes or more than one of these.

15 Next, the patient is administered one or more than one dose of a plasmid construct according to the present invention. That is, a plasmid construct comprising a polynucleotide encoding an autoantigen for the autoimmune disease and encoding BAX, or a plasmid construct comprising a polynucleotide encoding E3-GP19k but without a polynucleotide encoding an autoantigen for the autoimmune disease, or a plasmid construct comprising a
20 polynucleotide encoding a truncated form of the anti-apoptotic protein BCL-2 but without a polynucleotide encoding an autoantigen for the autoimmune disease. In a preferred embodiment, the organism is administered two plasmid constructs according to the present invention. In a particularly preferred embodiment, the organism is administered all three plasmid constructs according to the present invention.

25 In a preferred embodiment, the plasmid construct is administered in a plurality of doses. In another preferred embodiment, the dose is between about 0.001 mg/Kg and about 10 mg/Kg. In another preferred embodiment, the dose is between about 0.01 mg/Kg and about 1 mg/Kg. In another preferred embodiment, the dose is about 0.05 mg/Kg. In a preferred embodiment, a suitable dose for a human adult is between about 0.5 mg and 5 mg.
30 In a preferred embodiment, a suitable dose for a human adult is between about 1 mg and 4 mg. In a preferred embodiment, a suitable dose for a human adult is between about 2.5 mg and 3 mg. In another preferred embodiment, the dose is administered weekly between about

2 and about 10 times. In a particularly preferred embodiment, the dose is administered weekly 4 times. In another particularly preferred embodiment, the dose is administered only once.

5 Administration can be by a suitable route. In a preferred embodiment, the route is intramuscular or intravenous.

Additionally, the method can comprise, after administering, monitoring the patient for the development of the autoimmune disease.

EXAMPLE I

PREVENTION OF DIABETES

10 According to the present invention, the onset of diabetes in a patient is delayed or prevented, for example, as follows. First, the patient is selected based on the presence of circulating anti-insulin and anti-GAD autoantibodies. Next, the patient is injected intramuscularly with 0.05 mg/Kg of a plasmid construct comprising a polynucleotide sequence, SEQ ID NO:1, encoding the pro-apoptotic protein BAX and encoding SGAD, SEQ 15 ID NO:6, or comprising a polynucleotide sequence, SEQ ID NO:2, encoding the adenoviral protein E3-GP19k, or comprising a polynucleotide sequence, SEQ ID NO:3, encoding ΔBCL-2. The injection is repeated weekly for 3 weeks while the level of circulating anti-insulin and anti-GAD autoantibodies is monitored. The treatment is ended when the level of circulating anti-insulin and anti-GAD autoantibodies has returned to normal.

20

Although the present invention has been discussed in considerable detail with reference to certain preferred embodiments, other embodiments are possible. Therefore, the scope of the appended claims should not be limited to the description of preferred embodiments contained in this disclosure.

WHAT IS CLAIMED IS:

1. A substance for preventing, delaying the onset of or treating one or more than one autoimmune disease, the substance comprising a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for the autoimmune disease.

5 2. Use of a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for an autoimmune disease for the manufacture of a medicament for preventing, delaying the onset of or treating the one or more than one autoimmune disease.

10 3. Use of a polynucleotide construct comprising a polynucleotide sequence encoding the adenoviral protein E3-GP19k for the manufacture of a medicament for preventing, delaying the onset of or treating one or more than one autoimmune disease.

15 4. Use of a polynucleotide construct comprising a polynucleotide sequence encoding ΔBCL-2 for the manufacture of a medicament for preventing, delaying the onset of or treating one or more than one autoimmune disease.

5 5. Use according to any one of claims 2 to 4, where the medicament is manufactured in dosage units of between about 0.5 mg to about 5 mg.

6 6. Use according to any one of claims 2 to 4, where the medicament is manufactured in dosage units of between about 1 mg to about 4 mg.

20 7. Use according to any one of claims 2 to 4, where the medicament is manufactured in dosage units of between about 2.5 mg to about 3 mg.

8 8. Use according to any one of claims 2 to 4, where the medicament is manufactured in a form suitable for intramuscular administration.

9 9. Use according to any one of claims 2 to 4, where the medicament is manufactured in a form suitable for intravenous administration.

25 10. A method for preventing, delaying the onset of or treating an autoimmune disease in a patient comprising:

a) selecting a patient who is susceptible to developing the autoimmune disease, who is developing the autoimmune disease or who has the autoimmune disease; and

30 b) administering to the patient one or more than one dose of a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for the autoimmune disease.

11. The method of claim 10, where the autoimmune disease is type I diabetes.
12. The method of claim 11, where selecting the patient comprises identifying in the patient the presence of anti-insulin or anti-GAD autoantibodies or both anti-insulin and anti-GAD autoantibodies.
- 5 13. The method of claim 11, where selecting the patient comprises identifying in the patient the presence of increasing hyperglycemia.
14. The method of claim 11, where selecting the patient comprises identifying in the patient the presence of glycosuria.
- 15 15. The method of claim 10, where selecting the patient comprises identifying in the patient the presence of a genetic predisposition to the autoimmune disease.
- 10 16. The method of claim 10, where the one or more than one dose is a plurality of doses.
17. The method of claim 10, where administering to the patient one or more than one dose comprises injecting the patient intramuscularly with the one or more than one dose.
- 15 18. The method of claim 10, further comprising, after administering, monitoring the patient for the development the autoimmune disease.
19. A method for preventing, delaying the onset of or treating an autoimmune disease in a patient comprising:
 - a) selecting a patient who is susceptible to developing the autoimmune disease, who is developing the autoimmune disease or who has the autoimmune disease; and
 - b) administering to the patient one or more than one dose of a polynucleotide construct comprising a polynucleotide sequence encoding the adenoviral protein E3-GP19k.
- 20 20. The method of claim 19, where the autoimmune disease is type I diabetes.
21. The method of claim 20, where selecting the patient comprises identifying in the patient the presence of anti-insulin or anti-GAD autoantibodies or both anti-insulin and anti-GAD autoantibodies.
- 25 22. The method of claim 20, where selecting the patient comprises identifying in the patient the presence of increasing hyperglycemia.
23. The method of claim 20, where selecting the patient comprises identifying in the patient the presence of glycosuria.
- 30 24. The method of claim 19, where selecting the patient comprises identifying in the patient the presence of a genetic predisposition to the autoimmune disease.

25. The method of claim 19, where the one or more than one dose is a plurality of doses.

26. The method of claim 19, where administering to the patient one or more than one dose comprises injecting the patient intramuscularly with the one or more than one dose.

5 27. The method of claim 19, further comprising, after administering, monitoring the patient for the development the autoimmune disease.

28. A method for preventing, delaying the onset of or treating an autoimmune disease in a patient comprising:

10 a) selecting a patient who is susceptible to developing the autoimmune disease, who is developing the autoimmune disease or who has the autoimmune disease; and

b) administering to the patient one or more than one dose of a polynucleotide construct comprising a polynucleotide sequence encoding ABCL-2.

29. The method of claim 28, where the autoimmune disease is type I diabetes.

15 30. The method of claim 29, where selecting the patient comprises identifying in the patient the presence of anti-insulin or anti-GAD autoantibodies or both anti-insulin and anti-GAD autoantibodies.

31. The method of claim 29, where selecting the patient comprises identifying in the patient the presence of increasing hyperglycemia.

20 32. The method of claim 29, where selecting the patient comprises identifying in the patient the presence of glycosuria.

33. The method of claim 28, where selecting the patient comprises identifying in the patient the presence of a genetic predisposition to the autoimmune disease.

34. The method of claim 28, where the one or more than one dose is a plurality of doses.

25 35. The method of claim 28, where administering to the patient one or more than one dose comprises injecting the patient intramuscularly with the one or more than one dose.

36. The method of claim 28, further comprising, after administering, monitoring the patient for the development the autoimmune disease.

30 37. A method for preventing, delaying the onset of or treating an autoimmune disease in a patient comprising:

a) selecting a patient who is susceptible to developing the autoimmune disease, who is developing the autoimmune disease or who has the autoimmune disease; and

b) administering to the patient one or more than one dose of a polynucleotide construct comprising a polynucleotide sequence encoding the pro-apoptotic protein BAX and encoding one or more than one autoantigen for the autoimmune disease, or a polynucleotide construct comprising a polynucleotide sequence encoding the adenoviral protein E3-GP19k, 5 or a polynucleotide construct comprising a polynucleotide sequence encoding ΔBCL-2, or a combination of the preceding polynucleotide constructs.

38. The method of claim 37, where the autoimmune disease is type I diabetes.
39. The method of claim 38, where selecting the patient comprises identifying in the patient the presence of anti-insulin or anti-GAD autoantibodies or both anti-insulin and anti-10 GAD autoantibodies.
40. The method of claim 38, where selecting the patient comprises identifying in the patient the presence of increasing hyperglycemia.
41. The method of claim 38, where selecting the patient comprises identifying in the patient the presence of glycosuria.
- 15 42. The method of claim 37, where selecting the patient comprises identifying in the patient the presence of a genetic predisposition to the autoimmune disease.
43. The method of claim 37, where the one or more than one dose is a plurality of doses.
44. The method of claim 37, where administering to the patient one or more than one dose comprises injecting the patient intramuscularly with the one or more than one dose. 20
45. The method of claim 37, further comprising, after administering, monitoring the patient for the development the autoimmune disease.

1/2

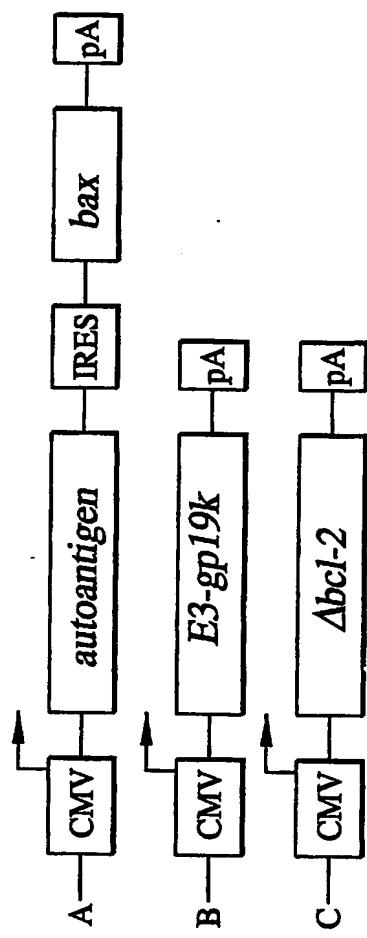
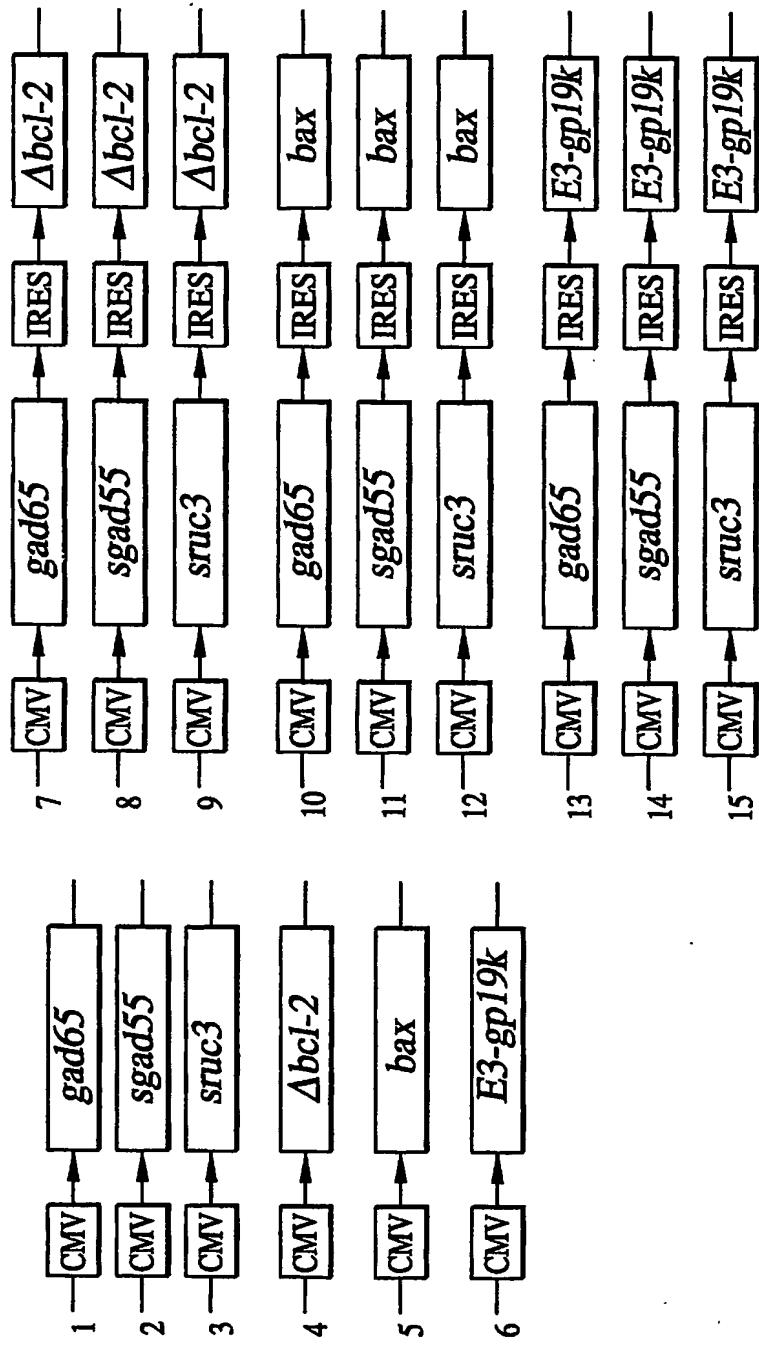


FIG.1

2/2

FIG.2



SEQUENCE LISTING

<110> Loma Linda University
ESCHER, Alan P.
LI, Fengchun

<120> Substances for Preventing and Treating Autoimmune Diseases

<130> 14102-1PCT

<140> to be assigned
<141> 2003-08-06

<150> US 60/401,652

<151> 2002-08-06

<160> 7

<170> PatentIn version 3.2

<210> 1
<211> 579
<212> DNA
<213> Homo sapiens

<400> 1
atggacgggt ccggggagca gcccagaggc ggggggcccc caagctctga gcagatcatg 60
aagacagggg ccctttgct tcagggtttc atccaggatc gagcagggcg aatggggggg 120
gaggcacccg agctggccct ggaccgggtg ctcaggatg cgtccaccaa gaagctgagc 180
gagtgtctca agcgcatcg ggacgaactg gacagtaaca tggagctgca gaggatgatt 240
gcgcgcgtgg acacagactc ccccgagag gtcttttcc gagtggcagc tgacatgttt 300
tctgacggca acttcaactg gggccgggtt gtcgccttt tctactttgc cagcaaactg 360
gtgctcaagg ccctgtgcac caaggtgccg gaactgatca gaaccatcat gggctggaca 420
ttggacttcc tccgggagcg gctgttgggc tggatccaag accagggtgg ttgggacggc 480
ctcctctcct actttggac gcccacgtgg cagaccgtga ccatcttgtt ggcgggagtg 540
ctcaccgcct cgctcaccat ctggaagaag atgggctga 579

<210> 2
<211> 492
<212> DNA
<213> Human adenovirus type 2

<400> 2
atgaggtaca tgattttagg cttgctcgcc cttgcggcag tctgcagcgc tgccaaaaag 60
gtttagttta aggaaccagc ttgcaatgtt acatttaaat cagaagctaa tgaatgcact
actcttataa aatgcaccac agaacatgaa aagcttatta ttgcaccaa agacaaaatt 120
ggcaagtatg ctgtatgc tatggcag ccaggtgaca ctaacgacta taatgtcaca
gtctccaag gtgaaaatcg taaaactttt atgtataaat ttccatTTt tgaaatgtgc 180
gatattacca tgtacatgag caaacagtc aagttgtggc ccccacaaaa gtgttagag 240
aacactggca cctttgttc caccgcctg cttattacag cgcttgctt ggtatgtacc
ttactttatc tcaaatacaa aagcagacgc agtttattt atgaaaagaa aatgccttga 300
tttccgctt gc 360
420
480
492

<210> 3
<211> 599
<212> DNA
<213> Homo sapiens

<400> 3
atggcgcacg ctgggagaag tggttacgat aaccgggaga tagtcatgaa gtacatccat 60
tataagctgt cgcaaggggg ctacgagtgg gatgctaccg cgctgccgc gggcctgcg 120
ctcagccgg tgccacctgt ggtccacctg accctccgccc aggccggcga cgacttctcc
cgccgctacc gccgcgactt cgccgagatg tccagccagc tgcacctgac gcccttcacc 180
gcgcggggat gcttgccac ggtggtagag gagctttca gggacgggggt gaactggggg 240
aggattgtgg cttttttga gttcggtgg gtcatgtgtg tggagagcgt caaccgggag 300
atgtcgcccc tggggacaa catgcctg tggatgactg agtacctgaa ccggcacctg
cacacctgga tccaggataa cggaggctgg gatgccttgc tggaaactgta cggccccagc 360
420
480

atgcggcctc tgtttgattt ctccctggctg tctctgaaga ctctgctcag tttggccctg 540
gtgggagctt gcatcacccct gggtgccatat ctgggccaca agtgaagtca acatgcctg 599

<210> 4
<211> 619
<212> DNA
<213> Encephalomyocarditis virus

<400> 4
tctagataat acgactcaact atagggcgaa ttccccctct ccctcccccc cccctaacgt 60
tactggccga agccgcttgg aataaggccg gtgtgcgttt gtctatatgt tattttccac 120
catattgccc agccgcttgg aataaggccg gtgtgcgttt gtctatatgt tattttccac 180
cattcctagg ggtctttccc ctctcgccaa aggaatgcaa ggctctgtga atgtcgtgaa 240
ggaaggcagtt cctctggaag cttcttgaag acaaacaacg tctgtagcga ccctttgcag 300
gcagcggAAC ccccccacctg gcgcacagggtg cctctgcggc caaaagccag gtgtataaga 360
tacacctgca aaggcggcac aaccccaagtg ccacgttgcg agttggaata gttgtggaaa 420
gagtcaaatg gctctcctca agcgtattca acaaggggct gaaggatgcc cagaaggtac 480
cccattgtat gggatctgat ctggggcctc ggtgcacatg ctttacatgt gtttagtcga 540
ggtaaaaaaa cgtctaggcc ccccaaccac ggggacgtgg ttttcccttg aaaaacacga 600
ttattatatt gcctctaga 619

<210> 5
<211> 1868
<212> DNA
<213> Homo sapiens

<400> 5
gagctccacc gcgggtggcg ccgctctaga ccaccatggc atctccgggc tctggcttt 60
ggtctttcgg gtcggaagat ggctctgggg attccgagaa tcccgccaca gcgcgagcct 120
ggtgccaagt ggctcagaag ttcacgggcg gcatcgaaaa caaactgtgc gccctgctct 180
acggagacgc cgagaagccg gcggagagcg gcgggagcca accccccgcgg gcccggccc 240

ggaaggccgc ctgcgcctgc gaccagaagc cctgcagctg ctccaaagtg gatgtcaact 300
acgcgtttct ccatgcaaca gacctgctgc cggcgtgtga tggagaaaagg cccactttgg 360
cgtttctgca agatgttatg aacattttac ttcaagtatgt ggtaaaaagt ttcaatagat 420
caacccaaagt gattgatttc cattatccta atgagcttct ccaagaatat aattggaaat 480
tggcagacca accacaaaat ttggaggaaa ttttgatgca ttgccaaaca actctaaaat 540
atgcaattaa aacagggcat cctagatact tcaatcaact ttctactggg ttggatatgg 600
ttggattagc agcagactgg ctgacatcaa cagcaaatac taacatgttc acctatgaaa 660
ttgctccagt atttgtgctt ttggaatatg tcacactaaa gaaaatgaga gaaatcattg 720
gctggccagg gggctctggc gatggatat tttctccgg tggcgccata tctaacatgt 780
atgccatgat gatgcacgc tttaagatgt tcccagaatgt caaggagaaa ggaatggctg 840
ctcttcccag gtcattgcc ttcacgtctg aacatagtca ttttctctc aagaagggag 900
ctgcagcctt agggattgga agagacagcg tgattctgat taaatgtgat gagagaggga 960
aaatgattcc atctgatctt gaaagaagga ttcttgaagc caaacagaaa gggtttgttc 1020
ctttctcgt gagtgccaca gctggAACCA ccgtgtacgg agcatttgac cccctcttag 1080
ctgtcgctga catttgcaaa aagtataaga tctggatgca tgtggatgca gcttgggttg 1140
ggggattact gatgtcccga aaacacaagt gggaaactgag tggcgtggag agggccaact 1200
ctgtgacgtg gaatcccacac aagatgtatgg gagtcccctt gcagtggctc gctctcctgg 1260
ttagagaaga gggattgtatg cagaattgca accaaatgca tgcctcctac ctcttcagc 1320
aagataaaaca ttatgacctg tcctatgaca ctggagacaa gcccttacag tgccggacgcc 1380
acgttcatgt ttttaaacta tggctgtatgt ggagggcaaa ggggactacc gggtttgaag 1440
cgcatgttga taaatgtttt gagggtggcag agtattttata caacatcata aaaaaccgag 1500
aaggatatga gatgggtttt gatgggaagc ctgaggacac aaatgtctgc ttctggataca 1560
ttcctccaag cttgcgtact ctggaaagaca atgaagagag aatgagtcgc ctctcgaagg 1620

tggctccagt gattaaagcc agaatgatgg agtatggAAC cacaatggTC agtaccaAC 1680
ccttggaga caaggtaat ttcttcgca tggcatctc aaaccCAGCG gcaactcacc 1740
aagacattGA cttcctgatt gaagaaATAG AACGCCTTGG ACAAGATTa TAATAACCTT 1800
gctcaccaAG ctgttccACT tctctAGGTA GCGACCTCGA GCGGCCGCTC gagggggggc 1860
ccggtaCC . 1868

<210> 6
<211> 1638
<212> DNA
<213> Artificial

<220>
<223> secreted form of human GAD

<400> 6
atgtacagga tgcaactcct gtcttgCATT gcactaAGTC ttgcacttgt cacaacAGT 60
gcacctaCTT acgcgtttCT ccatgcaACA gacctgctgc cggcgtgtga tggagaaAGG 120
cccactttgg cgTTTCTGCA agatgttATG aacattttAC ttcaGATGT ggtaaaaAGT 180
ttcgatAGAT caaccaaAGT gattgattTC cattatCCTA atgagCTTCT ccaagaATAT 240
aattggaaAT tggcagacca accacaaaAT ttggaggAAA ttttgatGCA ttgcAAACA 300
actctaaaAT atgcaattAA aacaggGCAT CCTAGATACT tcaatcaACT ttctactGGT 360
ttggatATGG ttggattAGC agcagactGG ctgacatCAA cagcaaatac taacatgtTC 420
acctatgAAA ttgctccAGT atttGtgCTT ttggaaATATG tcacactAAA gaaaATGAGA 480
gaaatcATTG gctggccAGG gggctctggc gatggatAT tttctccGG tggcgccATA 540
tctaACATGT atgCCATGAT gatcgCACGC ttaAGATGT tccCAGAAGT caaggAGAAA 600
ggaatggCTG ctcttcccAG gctcattGCC ttcacgtctG aacatAGTC ttttctCTC 660
aagaagggAG ctgcAGCCTT agggattGGA agagacAGCG tgattctGAT taaatgtGAT 720
gagagagggA aaatgattCC atctgatCTT gaaAGAAGGA ttcttGAAGC caaacAGAAA 780
gggtttgtTC ctTtcctcGT gagtGCCACA gctggAACCA ccgtgtacGG agcatttgAC 840

ccctcttag ctgtcgctga catttgcaaa aagtataaga tctggatgca tgtggatgca	900
gttgggggtg ggggattact gatgtcccga aaacacaagt ggaaactgag tggcgtggag	960
aggccaact ctgtgacgtg gaatccacac aagatgatgg gagtccttt gcagtggct	1020
gctctcctgg ttagagaaga gggattgatg cagaattgca accaaatgca tgccctcctac	1080
ctcttcagc aagataaaca ttatgacctg tcctatgaca ctggagacaa ggccttacag	1140
tgcggacgcc acgttgatgt ttttaaacta tggctgatgt ggagggcaaa gggactacc	1200
gggttgaag cgcatgttga taaatgtttg gagttggcag agtatttata caacatcata	1260
aaaaaccgag aaggatatga gatggtgttt gatgggaagc ctgaggacac aaatgtctgc	1320
ttctggtaca ttccctccaag cttgcgtact ctggaagaca atgaagagag aatgagtcgc	1380
ctctcgaagg tggctccagt gattaaagcc agaatgatgg agtatggaac cacaatggc	1440
agctaccaac cttgggaga caaggtcaat ttcttccgca tggcatctc aaacccagcg	1500
gcaactcacc aagacattga cttcctgatt gaagaaatag aacgccttgg acaagattt	1560
taataacctt gtcaccaag ctgttccact tctctaggta gcgacctcga gcggccgctc	1620
gaaaaaaaaa ccgttacc	1638

<210> 7
<211> 1271
<212> DNA
<213> Artificial

<220>
<223> secreted form of Renilla luciferase

<400> 7 atgtacagga tgcaactcct gtcttgcatt gcactaagtc ttgcacttgt cacaacagt	60
gcacctactg aattcagctt aaagatgact tcgaaagttt atgatccaga acaaaggaaa	120
cggatgataa ctggtccgca gtgggtggcc agatgtaaac aaatgaatgt tcttgattca	180
tttattaatt attatgattc agaaaaacat gcagaaaaatg ctgttatttt tttacatggt	240

aacgcggcct cttcttattt atggcgacat gttgtgccac atattgagcc agtagcgcgg 300
tgtattatac cagatcttat tggtatggc aaatcaggca aatctggtaa tggttcttat 360
aggttacttg atcattacaa atatcttact gcatggtttg aacttcttaa tttaccaaag 420
aagatcattt ttgtcggcca tgattgggt gctgcttgg catttcatta tagctatgag 480
catcaagata agatcaaagc aatagttcac gctgaaaagtg tagtagatgt gattgaatca 540
tggatgaat ggcctgatat tgaagaagat attgcgttga tcaaatctga agaaggagaa 600
aaaatggttt tggagaataa cttcttcgtg gaaaccatgt tgccatcaaa aatcatgaga 660
aagttagaac cagaagaatt tgcagcatat cttgaaccat tcaaagagaa aggtgaagtt 720
cgtcgtccaa cattatcatg gcctcgtgaa atcccgttag taaaaggtgg taaacctgac 780
gttgcataaa ttgttaggaa ttataatgct tatctacgtg caagtgtatga tttaccaaaa 840
atgtttattt aatcgatcc aggattctt tccaatgcta ttgttgaagg cgccaagaag 900
tttcctaata ctgaatttgc caaagtaaaa ggtcttcatt ttgcgaaga agatgcaccc 960
gatgaaatgg gaaaatatat caaatcggtc gttgagcgag ttctcaaaaa tgaacaataa 1020
ttacttttgtt ttttattta cattttccc gggtttaata atataaatgt cattttcaac 1080
aattttattt taactgaata ttccacaggg aacattcata tatgttgatt aatttagctc 1140
gaactttact ctgtcatatc attttggaaat attaccttt tcaatgaaac tttataaaca 1200
gtggttcaat taattaatat atattataat tacatttgc ttgtataaaa ctggtttta 1260
ttataaaaaa a 1271